AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.-38. (Canceled).

39. (New) A single-container method for synthesizing cDNA from RNA in a biological sample using a reverse transcriptase and amplifying and detecting at least one selected sequence of said cDNA, comprising:

a) combining said biological sample with a disruption reagent containing a chaotropic salt having a concentration of at least 2M that denatures or degrades all proteins, including nucleases, to produce a disrupted sample containing RNA freed from bound proteins and inactivating nucleases with no more than twenty-five percent loss of RNA;

b) reducing the concentration of chaotropic salt in said disrupted sample to less than 0.05M by diluting without washing the disrupted sample with at least one aqueous reagent before adding the reverse transcriptase;

c) incubating the diluted, disrupted sample with reverse transcriptase to transcribe said RNA to cDNA;

d) amplifying at least one selected sequence of said cDNA; and

e) detecting the at least one amplified cDNA sequence

wherein steps b) through e) are performed without first separating the RNA from degraded proteins or from said chaotropic salt.

40. (New) The method according to claim 39, wherein said disruption reagent includes a cell-lysing detergent.

41. (New) The method according to claim 40, wherein said biological sample comprises at least one cell.

42. (New) The method of claim 39, wherein step a) includes heating to concentrate the chaotropic salt after combination of the biological sample with the disruption reagent.

43. (New) The method of claim 42, wherein the initial concentration of said chaotropic salt is at least 2M.

44. (New) The method of claim 42, wherein said heating is sufficient to produce a disrupted sample that is at least semi-dry.

45. (New) The method of claim 44, wherein said disruption reagent includes a water-miscible solvent that prevents precipitation of the chaotropic salt and that evaporates during said heating.

46. (New) The method of claim 39, wherein the chaotropic salt is a dry reagent that is dissolved in the biological sample.

47. (New) The method of claim 39, wherein the container is a microfluidic device.

48. (New) The method of claim 39, wherein in step a) the concentration of chaotropic salt is reduced to less than 0.01M.

49. (New) The method of claim 48, wherein the chaotropic salt is a dry reagent that is dissolved in the biological sample.

50. (New) The method of claim 49, wherein the biological sample includes phosphate buffered saline (PBS).

51. (New) The method of claim 49, wherein said dry reagent is adhered to a surface of the container.

52. (New) The method of claim 51, wherein said surface is the inner surface of a tube, a tube cap, a wall of a microtiter plate of a microtiter plate cover.

53. (New) The method of claim 49, wherein the chaotropic salt has a concentration of at least 2M when dissolved in the biological sample.

54. (New) The method of claim 53, wherein step a) includes heating to concentrate the chaotropic salt after combination of the biological sample with the disruption reagent.

55. (New) The method of claim 39, wherein step a) includes heating to concentrate the chaotropic salt after combination of the biological sample with the disruption reagent.

56. (New) The method of claim 55, wherein step b) includes diluting with water containing random hexamers, heating to denature double strands, and cooling to anneal the random hexamers to the RNA.

57. (New) The method of claim 56, wherein step c) comprises adding reverse transcriptase and RT buffer in an amount that does not further dilute the disrupted, diluted sample from step b) by more than a factor of six.

58. (New) The method of claim 57, wherein in step d) amplification is carried out in amplification buffer added in this step and wherein that addition dilutes the cDNA by a factor of at least nine.

59. (New) The method of claim 54, wherein the initial concentration of chaotropic salt is at least 2M.

60. (New) The method of claim 59, wherein said heating is sufficient to produce a disrupted sample that is at least semi-dry.

61. (New) The method of claim 60, wherein said disruption reagent includes a water-miscible solvent that prevents precipitation of the chaotropic salt and that evaporates during said heating.

62. (New) The method of claim 61, wherein the solvent is DMSO.

63. (New) The method of claim 61, wherein said disruption reagent includes a cell-lysing detergent.

64. (New) The method of claim 63, wherein said biological sample contains at least one cell.

65. (New) The method of claim 60, wherein step b) includes diluting progressively with three aqueous solutions : a first solution containing DNase, a second solution containing a

chelating agent, and a third solution containing random hexamers, and wherein the container is heated following addition of the chelating agent.

66. (New) The method of claim 39 wherein amplification and detection reagents are added in step c) and wherein step c) further dilutes the disrupted, diluted sample by at least a factor of nine.